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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07H 21/00

A1

(11) International Publication Number: WO 98/20019

(43) International Publication Date: 14 May 1998 (14.05.98)

(21) International Application Number: PCT/US97/20194

(22) International Filing Date: 6 November 1997 (06.11.97)

(30) Priority Data:

08/746,036	6 November 1996 (06.11.96)	US
08/786,988	23 January 1997 (23.01.97)	US
08/787,639	23 January 1997 (23.01.97)	US
08/933,792	19 September 1997 (19.09.97)	US

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

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Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMPOSITIONS AND METHODS FOR IMMOBILIZING NUCLEIC ACIDS TO SOLID SUPPORTS

(57) Abstract

Compositions containing at least one bead conjugated to a solid support and further conjugated to at least one macromolecule, such as a nucleic acid and methods for making the compositions are provided. The resulting surfaces formed from the beads linked to the solid support advantageously provide increased surface area for immobilization of biological particles or macromolecules, particularly nucleic acids compared to "flat" surfaces. Furthermore, by selecting a bead with the desired functionality, an appropriate functionalization chemistry for immobilizing the selected macromolecule or biological support, which is different from the chemistry of the solid support, can be produced.

COMPOSITIONS AND METHODS FOR IMMOBILIZING NUCLEIC ACIDS TO SOLID SUPPORTS

RELATED APPLICATIONS

For U.S. purposes, this application is a continuation-in-part of U.S. application Serial No. 08/933,792, filed September 19, 1997, entitled "COMPOSITIONS AND METHODS FOR IMMOBILIZING NUCLEIC ACIDS TO SOLID SUPPORTS", which is a continuation-in-part of U.S. application Serial No. 08/746,036 filed November 6, 1996, entitled "BEAD LINKERS FOR IMMOBILIZING NUCLEI ACID TO SOLID SUPPORTS". This application is also a continuation-in-part U.S. application Serial No. 08/746,036.

For international purposes benefit of priority to each of these applications is claimed herein. Benefit of priority is also claimed to U.S. application Serial Nos. 08/786,988 and 08/787,639, each filed on January 23, 1997.

Where permitted, the subject matter of each of U.S. application Serial Nos. 08/933,792, 08/746,036, 08/786,988 and 08/787,639 are herein incorporated in their entirety by reference thereto.

BACKGROUND OF THE INVENTION

In the fields of molecular biology and biochemistry, as well as in the diagnosis of diseases, nucleic acid hybridization has become a powerful tool for the detection, isolation, and analysis of specific oligonucleotide sequences. Typically, such hybridization assays utilize an oligodeoxynucleotide probe that has been immobilized on a solid support; as for example in the reverse dot blot procedure (Saiki et al. (1989) Proc. Natl. Acad. Sci. U.S.A. 86:6230). More recently, arrays of immobilized DNA probes attached to a solid surface have been developed for sequencing by hybridization (SBH) (Drmanac et al. (1989) Genomics 4:114-128), (Strezoska et al. (1991) Proc. Natl. Acad. Sci.

Acids Res. 22:2121-2125; Eggers et al. (1994) BioTechniques 17:516-524) or amino groups (see, e.g., Rasmussen et al. (1991) Anal. Biochem. 198:138-142). Although many of these methods were successful for their respective applications, when used to link nucleic acids to two-dimensional (flat) supports, the density of the immobilized oligodeoxynucleotide is often insufficient for the ensuing analyses (see, e.g., Lamture (1994) Nucl. Acids Res. 22:2121-2125; Eggers et al. (1994) BioTechniques 17:516-524).

Thus, there is a need for improved methods for immobilization that provide higher densities of linked molecules for ensuing analyses. Therefore, it is an object herein to provide methods for preparing solid supports containing high densities of immobilized molecules, particularly nucleic acid molecules.

SUMMARY OF THE INVENTION

Compositions containing at least one bead conjugated to a solid support and further conjugated to at least one molecule, particularly a nucleic acid are provided. The bead is formed from any suitable matrix material known to those of skill in the art, including those that are swellable and nonswellable. The solid support is any support known to those of skill in the art for use as a support matrix in chemical syntheses and analyses.

Preferably the bead is made of a material selected from materials that serve as solid supports for synthesis and for assays including but not limited to: silica gel, glass, magnet, polystyrene/1% divinylbenzene resins, such as Wang resins, which are Fmoc-amino acid-4-(hydroxy-methyl)phenoxymethylcopoly(styrene-1% divinylbenzene (DVD)) resin, chlorotrityl (2-chlorotritylchloride copolystyrene-DVB resin) resin, Merrifield (chloromethylated copolystyrene-DVB) resin metal, plastic, cellulose, cross-linked dextrans, such as those sold under the tradename

The above and further features and advantages of the compositions and methods provided herein will be elucidated in the following Figures, Detailed Description and Claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic showing the covalent attachment of a bead to a solid support and DNA to the bead.

Figure 2 is a schematic showing the covalent attachment of Wang resin beads (p-benzyloxybenzyl alcohol copolystyrene-divinyl benzene (DVB) resin) to a solid support as described in Example 1.

Figure 3 is a schematic representation of nucleic acid immobilization via covalent bifunctional trityl linkers as described in Example 2.

Figure 4 is a schematic representation of nucleic acid immobilization via hydrophobic trityl linkers as described in Example 3.

Figure 5 shows a MALDI-TOF mass spectrum of a supernatant of the matrix treated Dynabeads containing bound oligo (5' iminobiotin-TGCACCTGACTC, SEQ. ID. No. 1). An internal standard (CTGTGGTCGTGC, SEQ. ID. No. 2) was included in the matrix.

Figure 6 shows a MALDI-TOF (matrix-assisted laser desorption/ionization (MALDI)-time-of-flight (TOF)) mass spectrum of a supernatant of biotin treated Dynabeads containing bound oligo (5' iminobiotin-TGCACCTGACTC, SEQ. ID. No. 1). An internal standard (CTGTGGTCGTGC, SEQ. ID. No. 2) was included in the matrix.

Figure 7 schematically depicts conjugation of an unextended primer to a bead via reaction of a 2', 3'-diol on the primer with boronic acid functionalized beads.

Figure 8 schematically depicts a pin tool apparatus.

Figure 9 depicts various pin conformations. Figures 9A shows a solid pin with a straight head. Figure 9B shows a solid pin with a

DETAILED DESCRIPTION OF THE INVENTION

A. Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which this invention belongs. All patents and publications referred to herein are, unless noted otherwise, incorporated by reference in their entirety. In the event a definition in this section is not consistent with definitions elsewhere, the definition set forth in this section will control.

As used herein, a molecule refers to any molecule or compound that is linked to the bead. Typically such molecules are macromolecules or components or precursors thereof, such as peptides, proteins, small organics, oligonucleotides or monomeric units of the peptides, organics, nucleic acids and other macromolecules. A monomeric unit refers to one of the constituents from which the resulting compound is built. Thus, monomeric units include, nucleotides, amino acids, and pharmacophores from which small organic molecules are synthesized.

As used herein, macromolecule refers to any molecule having a molecular weight from the hundreds up to the millions. Macromolecules include peptides, proteins, nucleotides, nucleic acids, and other such molecules that are generally synthesized by biological organisms, but can be prepared synthetically or using recombinant molecular biology methods.

As used herein, a biological particle refers to a virus, such as a viral vector or viral capsid with or without packaged nucleic acid, phage, including a phage vector or phage capsid, with or without encapsulated nucleotide acid, a single cell, including eukaryotic and prokaryotic cells or fragments thereof, a liposome or micellar agent or other packaging particle, and other such biological materials. For purposes herein,

In preferred embodiments, the beads are functionalized for the immobilization of nucleic acids and are stably associated with a solid support. Figure 1 depicts a bead conjugated to a solid support through one or more covalent or non-covalent bonds. Nucleic acids can be immobilized on the functionalized bead before, during or after the bead is conjugated to the solid support.

Preferred nucleic acids for use herein are derivatized to contain at least one reactive moiety. Preferably the reactive moiety is at the 3′ or 5′ end. Alternatively, a nucleic acid can be synthesized with a modified base. In addition, modification of the sugar moiety of a nucleotide at positions other than the 3′ and 5′ position through conventional methods is contemplated. Also, nucleic acid bases can be modified, e.g., by using N7- or N9- deazapurine nucleosides or by modification of C-5 of dT with a linker arm (see, e.g., Eckstein, ed., "Oligonucleotides and Analogues: A Practical Approach," IRL Press (1991)). Alternatively, backbone-modified nucleic acids (e.g., phosphoroamidate DNA) can be used so that a reactive group can be attached to the nitrogen center provided by the modified phosphate backbone.

In preferred embodiments, modification of a nucleic acid, e.g., as described above, does not substantially impair the ability of the nucleic acid or nucleic acid sequence to hybridize to its complement. Thus, any modification should preferably avoid substantially modifying the functionalities of the nucleic acid which are responsible for Watson-Crick base pairing. The nucleic acid can be modified such that a non-terminal reactive group is present, and the nucleic acid, when immobilized to the support, is capable of self-complementary base pairing to form a "hairpin" structure having a duplex region.

Conjugation

The biological particles and macromolecules, such as nucleic acid molecules can be attached directly to the beads via a linker. Conjugation can be through any suitable means, particularly covalent or non-covalent attachment. For example, in one embodiment for conjugating nucleic acids to beads, the conjugating means introduces a variable spacer between the beads and the nucleic acids. In another embodiment, the conjugation is directly cleavable, such as a photocleavable linkage (e.g. streptavidin- or avidin- to biotin interaction can be cleaved by a laser, for example for mass spectrometry) or indirectly via photocleavable linker (see, e.g., U.S. Patent No. 5,643,722) or acid labile linker, heat sensitive linker, enzymatically cleavable linker or other such linker.

Similarly the bead is conjugated to the solid support by any suitable means, including those discussed herein for attachment of nucleic acids to beads. Thus, any of the conjugation methods and means discussed below with reference to conjugation of nucleic acids to beads can be applied for conjugation of beads to the solid support. In addition, it is understood that nucleic acids are exemplary of the molecules that can be conjugated to beads.

Conjugation via Linkers

Appropriate cross-linking agents for use for conjugating molecules to supports and beads and/or the beads to the supports include a variety of agents that are capable of reacting with a functional group present on a surface of the bead, insoluble support and or molecule, such as nucleic acid, and with a functional group present in the molecule, such as the nucleic acid and/or bead, respectively. Reagents capable of such reactivity include homo- and hetero-bifunctional reagents, many of which are known in the art. Heterobifunctional reagents are preferred. A preferred bifunctional cross-linking agent is N-succinimidyl(4-iodoacetyl)

e.g., formic acid, trifluoracetic acid, etc.) to ensure that the nucleic acid is cleaved and can be removed. In which case, the nucleic acid may be deposited as a beadless patch at the bottom of a well in the solid support or on the flat surface of the solid support. After addition of matrix solution, the nucleic acid can then be desorbed, for example into a mass spectrometer.

Hydrophobic trityl linkers can also be exploited as acid-labile linkers by using a volatile acid or an appropriate matrix solution (e.g. a matrix solution containing, for example, 3-hydroxypicolinic acid (3-HPA) to cleave the aminolink trityl group from the nucleic acid molecule). Also, the acid lability can be changed. For example, trityl, monomethoxy, dimethoxy- or trimethoxytrityl can be changed to the appropriate p-substituted and even more acid labile tritylamine derivatives of the nucleic acids (i.e. trityl ether and tritylamine bonds to the nucleic acid can be made). Therefore, the nucleic acid may be removed from the hydrophobic linker, for example, by disrupting the hydrophobic attraction or by cleaving tritylether or tritylamine bonds under acidic or the usual mass spectrometry conditions (e.g. wherein the matrix, such as 3-HPA acts as an acid).

In a particularly preferred embodiment the bead is conjugated to the solid support and/or the nucleic acid is conjugated to the bead using an acid-labile bond. For example, use of a trityl linker, as further described in the following Examples 2 and 3, can provide a covalent or hydrophobic conjugation. Regardless of the nature of the conjugation, the trityl group is readily cleaved in acidic conditions.

complementary nucleic acid, antibody-antigen and other such pairs know to those of skill in the art.

Examples of preferred binding pairs or linker/interactions is shown in the following Table 1:

TABLE 1

LINKER/INTERACTION	EXAMPLES
streptavidin-biotin[a,c]/photolabile biotin[b]	biotinylated pin, avidin beads, photolabile biotin DNA
hydrophobic[a]	C18-coated pin, tritylated DNA
magnetic[a]	electromagnetic pin, steptavidin magnetic beads (e.g., DYNABEADS), biotin DNA
acid-labile linker[b]	glass pin, bifunctional trityl-linked DNA
amide bond(s)[c]	silicon wafer, Wang resin, amino-linked DNA
disulfide bond[a]	silicon wafer, beads are bound on the flat surface forming arrays or in arrays of nanoliter wells, thiol beads, thiolated DNA
photocleavable bond/linker	biotinylated pin/wafer, avidin beads, photolabile biotin DNA
thioether bond[c]	silicon wafer, beads are bound on the flat surface forming arrays or in arrays of nanoliter wells, thiolated DNA

[a]These interactions are reversible. [b]These non-reversible interactions are rapidly cleaved. [c]Unless cleavable-linkers are incorporated at some point in the scheme, only the complement of the solid-bound DNA can be analyzed in these schemes. [d]Beads are bound on the

In a particularly preferred embodiment the bead is conjugated to the solid support and/or the nucleic acid is conjugated to the bead using an acid-labile bond. For example, use of a trityl linker, as further described in the following Examples 2 and 3, can provide a covalent or hydrophobic conjugation. Regardless of the nature of the conjugation,

The solid supports with beads and immobilized molecules can be used in any application for which solid supports with immobilized molecules are used. For example, the methods and compositions described herein, can be used to isolate (purify) target nucleic acids from biological samples (reactions). For example, the compositions and methods can be used to isolate particular nucleic acids, which are generated by cloning (Sambrook et al., Molecular Cloning : A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989), polymerase chain reaction (PCR) (C.R. Newton and A. Graham, PCR, BIOS Publishers, 1994), ligase chain reaction (LCR) (see, e.g., Wiedmann et al. (1994) PCR Methods Appl. 3:57-64; Barany (1991) Proc. Natl. Acad. Sci. U.S.A. 88:189-93), strand displacement amplification (SDA) (see, e.g., Walker et al. (1994) Nucl. Acids Res. 22:2670-77; European Patent Publication Number 0 684 315 entitled "Strand Displacement Amplification Using Thermophilic Enzymes") and variations such as reverse transcriptase (RT)-PCR (Higuchi et al. (1993) Bio/Technology 11:1026-1030), allele-specific amplification (ASA), cycle sequencing and transcription based processes.

Further the methods and compositions can be used to isolate or transfer particular nucleic acids during the performance of a particular reaction. For example, an amplification reaction, such as a PCR reaction, can be performed to 'master' mix without addition of the dideoxynucleotides (d/ddNTPs) or sequencing primers. Aliquots can be isolated via a conjugation means described herein and transferred, for example to a sequencing plate, where d/ddNTPs and primers can then be added to perform a sequencing reaction. Alternatively, the PCR can be split between A, C, G, and T master mixes. Aliquots can then be transferred to a sequencing plate and sequencing primers added.

specificity, the pin tool (with or without voltage), can be modified to contain a partially or fully single stranded oligonucleotide (e.g., about 5-12 base pairs). Only complementary nucleic acid sequences (e.g. in solution) are then specifically conjugated to the pins.

In yet a further embodiment, a PCR primer can be conjugated to the tip of a pin tool. PCR can be performed with the solid phase (pin tool)-bound primer and a primer in solution, so that the PCR product becomes attached to the pin tool. The pin tool with the amplification product can then be removed from the reaction and analyzed (e.g., by mass spectrometry).

Examples of different pin conformations are shown in Figure 9. For example, Figures 9a, 9b and 9c show a solid pin configuration. Figures 9d and 9e show pins with a channel or hole through the center, for example to accommodate an optic fiber for mass spectrometer detection. The pin can have a flat tip or any of a number of configurations, including nanowell, concave, convex, truncated conic or truncated pyramidal (e.g. size 4-800μ across x 100μ depth). In a preferred embodiment, the individual pins, which can be any desired size, are preferably up to about 10 mm in length, and more preferably are about 5 mm in length and about 1 mm in diameter. The pins and mounting plate can be made of polystyrene (e.g. one-piece injection molded). Polystyrene is an ideal material to be functionalized and can be molded with very high tolerances. The pins in a pin tool apparatus may be collapsible (e.g., controlled by a scissor-like mechanism), so that pins may be brought into closer proximity, reducing the overall size.

Captured nucleic acids can be analyzed by any of a variety of means including, for example, spectrometric techniques such as UV/VIS, IR, fluorescence, chemiluminescence, or NMR spectroscopy, mass spectrometry, or other methods known in the art, or combinations

Two mass spectrometer geometries for accommodating the pin tool apparatus (see Figure 9) are proposed in Figure 13. The first accommodates solid pins. In effect, the laser ablates a layer of material from the surface of the crystals, the resultant ions being accelerated and focused through the ion optics. The second geometry accommodates fibre optic pins in which the samples are lasered from behind. In effect, the laser is focused onto the pin tool back plate and into a short optical fibre (about 100 μm in diameter and about 7 mm length to include thickness of the back plate). This geometry requires the volatilized sample to go through the depth of the matrix/bead mix, slowing and cooling down the ions resulting in a type of delayed extraction which should actually increase the resolution of the analysis.

The probe through which the pins are fitted can also be of various geometries. For example, a large probe with multiple holes, one for each pin, fitted over the pin tool. The entire assembly is translated in the X-Y axes in the mass spectrometer. Alternatively, as a fixed probe with a single hole, which is large enough to give an adequate electric field, but small enough to fit between the pins. The pin tool is then translated in all three axes with each pin being introduced through the hole for sequential analyses. This format is more suitable for the higher density pin tool (i.e. based on a 384 well or higher density microplate format). The two probes described above, are suitable for the two mass spectrometer geometries described above.

Figure 14 schematically depicts the steps involved in mass spectrometry sequencing by post biology capture as described above.

Preparation of arrays that include beads in a flat surface or in wells

The methods provided herein are useful for providing spatially-addressable arrays of nucleic acids immobilized on beads, which are

disposes the sample arrays for analysis by mass spectrometry. A mass spectrometer is provided that generates a set of spectra signal that is indicative of the composition of the sample material under analysis.

In particular, the pin tool includes a housing having a plurality of sides and a bottom portion having formed therein a plurality of apertures, the walls and bottom portion of the housing defining an interior volume; one or more fluid transmitting vesicles, or pins, mounted within the apertures, having a nanovolume sized fluid holding chamber for holding nanovolumes of fluid, the fluid holding chamber being disposed in fluid communication with the interior volume of the housing, and a dispensing element that is in communication with the interior volume of the housing for selectively dispensing nanovolumes of fluid form the nanovolume sized fluid transmitting vesicles when the fluid is loaded with the fluid holding chambers of the vesicles. This allows the dispensing element to dispense nanovolumes of the fluid onto the surface of the substrate when the apparatus is disposed over and in registration with the substrate.

In one embodiment, the fluid transmitting vesicle has an open proximal end and a distal tip portion that extends beyond the housing bottom portion when mounted within the apertures. In this way the open proximal end can dispose the fluid holding chamber in fluid communication with the interior volume when mounted with the apertures. Optionally, the plurality of fluid transmitting vesicles are removably and replaceably mounted within the apertures of the housing, or alternatively can include a glue seal for fixedly mounting the vesicles within the housing.

In another embodiment, the fluid holding chamber includes a narrow bore dimensionally adapted for being filled with the fluid through capillary action, and can be sized to fill substantially completely with the

housing at varying pressure conditions. This allows the controller varying element to dispose the interior volume at a selected pressure condition sufficient to offset the capillary action to fill the fluid holding chamber of each vesicle to a predetermined height corresponding to a predetermined fluid amount. Additionally, the controller can further include a fluid selection element for selectively discharging a selected nanovolume fluid amount from the chamber of each the vesicle. In one particular embodiment, the a pressure controller that operates under the controller of a computer program operating on a data processing system to provide variable control over the pressure applied to the interior chamber of the housing is provided.

The fluid transmitting vesicle can have a proximal end that opens onto the interior volume of the housing, and the fluid holding chamber of the vesicles are sized to substantially completely fill with the fluid through capillary action without forming a meniscus at the proximal open end. Optionally, the apparatus can have plural vesicles, wherein a first portion of the plural vesicles include fluid holding chambers of a first size and a second portion including fluid holding chambers of a second size, whereby plural fluid volumes can be dispensed.

In another embodiment, the tool can include a fluid selection element that has a pressure source coupled to the housing and in communication with the interior volume for disposing the interior volume at a selected pressure condition, and an adjustment element that couples to the pressure source for varying the pressure within the interior volume of the housing to apply a positive pressure in the fluid chamber of each the fluid transmitting vesicle to vary the amount of fluid dispensed therefrom. The selection element and adjustment element can be computer programs operating on a data processing system that directs the operation of a pressure controller connected to the interior chamber.

Kits

Also provided is a kit for immobilizing nucleic acids on beads, which are further bound. In one embodiment, the kit includes an appropriate amount of: i) beads, and/or ii) the insoluble support, and iii) conjugation means. The kits described herein can also optionally include appropriate buffers; containers for holding the reagents; and/or instructions for use.

The following examples are included for illustrative purposes only and are not intended to limit the scope of the invention.

EXAMPLE 1

Attachment of Resin Beads to a Silicon Surface

A silicon surface (e.g. of a silicon wafer) is derivatized with amino groups by treatment with 3-aminopropyltriethoxysilane. Wang resin beads are treated with succinic anhydride to provide carboxyl-functionalized resin beads. The carboxyl-functionalized resin beads are then coupled to the amino-functionalized silicon surface with a coupling reagent (for example, dicyclohexylcarbodiimide (DCC)), in the presence of p-nitrophenol. The resin beads become covalently linked to the silicon surface, and the unreacted carboxyl groups of the resin are converted to the p-nitrophenyl ester (an activated ester suitable for coupling with a nucleic acid).

Alternatively, the carboxyl groups of the Wang resin are transformed to the p-nitrophenyl active esters prior to reacting with the amino-functionalized silicon surface.

Thus, resin beads can be rapidly and conveniently attached to a silicon surface, and can be simultaneously converted to a reactive form suitable for covalent attachment of nucleic acids.

Alternatively C18 beads were first covalently attached to a silicon surface (e.g. a silicon wafer) or adsorbed to a flat surface by hydrophobic interaction.

The results showed that acetonitrile/water at levels of ca. > 30% are enough to dissociate the hydrophobic interaction. Since the matrix used in MALDI contains 50% acetonitrile, the DNA can be released from the support and MALDIed successfully (with the trityl group removed during the MALDI process).

EXAMPLE 4

Attaching Beads to Silicon Chips

Amino derivatization of silicon surface

Silicon wafers were washed with ethanol to remove surface debris and flamed over a bunsen burner until "red hot" to ensure oxidation of the surface. After cooling, the wafers were immersed in an anhydrous solution of 3-aminopropyltriethoxysilane in toluene (25%v/v) for 3 hours. The wafers were then washed with toluene (three times) then anhydrous dimethylacetamide (three times).

Activation of Wang resin beads

Vacuum-dried Wang resin beads (5 g, 0.84 mmol/g loading, 4.2 mmol, diameter 100-200 mesh), obtained from Novabiochem, were suspended in pyridine (40 ml) with DMAP (0.1 eq, 0.42 mmol, 51 mg). To this was added succinic anhydride (5 eq, 21 mmol, 2.10 g) and the reaction was shaken for 12 hours at room temperature. After this time, the beads were washed with dimethylformamide (three times), then pyridine (three times) and suspended in pyridine/dimethylformamide (1:1, 20 ml). 4-Nitrophenol (2 eq, 8.4 mmol, 1.40 g) was added and the condensation was activated by adding dicyclohexylcarbodiimide (DCC) (2 eq, 8.4 mmol, 1.73 g) and the reaction mixture was shaken for 12

and 0.5 μl of supernatant was applied to MALDI-TOF MS. To maximize the recovery of the bound iminobiotin oligo, the beads from the above treatment were again incubated with 2 μl of 3-HPA matrix and 0.5 μl of the supernatant was applied to MALDI-TOF MS.

Matrix alone and free biotin treatment quantitatively released iminobiotin oligo off the streptavidin beads as shown in Figures 5 and 6. Almost no bound oligo was observed after the second treatment which confirmed the complete recovery.

Equivalents

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures described herein. Such equivalents are considered to be within the scope of this invention and are covered by the following claims.

Since modifications will be apparent to those of skill in this art, it is intended that this invention be limited only by the scope of the appended claims.

(D) SOFTWARE: NONE

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 06-NOV-1997
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/746,036
(B) FILING DATE: 11/06/96

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 08/933,792
(B) FILING DATE: 03/18/96

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(C) REFERENCE/DOCKET NUMBER: 7352-2003PC

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNES3: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TGCACCTGAC TC 12

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: DNA
(iii) HYPOTHETICAL: NO
(iv) ANTISENSE: NO
(v) FRAGMENT TYPE:
(vi) ORIGINAL SOURCE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTGTGGTCGT GC 12

11. A process of making a bead conjugated to a solid support and further conjugated to a macromolecule or biological particle, comprising:

(a) conjugating a bead to a macromolecule or biological particle; and

(b) conjugating a bead to a solid support, wherein steps (a) and (b) are performed sequentially in any order or simultaneously.

12. A process of claim 11, wherein the macromolecule is selected from the group consisting of nucleic acids, peptides, proteins, amino acids and organic molecules.

13. A process of claim 11, wherein the bead is conjugated to a macromolecule and the macromolecule is a nucleic acid.

14. A process of any of claims 11-13, wherein the bead is functionalized for linkage of macromolecules.

15. A process of any of claims 11-13, wherein the bead is functionalized with carboxy functional groups.

16. A process of any of claims 11-13, wherein the bead is functionalized with amino functional groups.

17. A process any of claims 11-13, wherein the bead is conjugated to the macromolecule or biological particle prior to conjugation of the bead to the solid support.

18. A process of claim 10, wherein the bead is conjugated to the macromolecule or biological particle after the bead is conjugated to the solid support.

19. A kit, comprising:

i) beads, and/or

ii) the insoluble support, and

iii) conjugation means for linking molecules to the beads and the beads to the support.

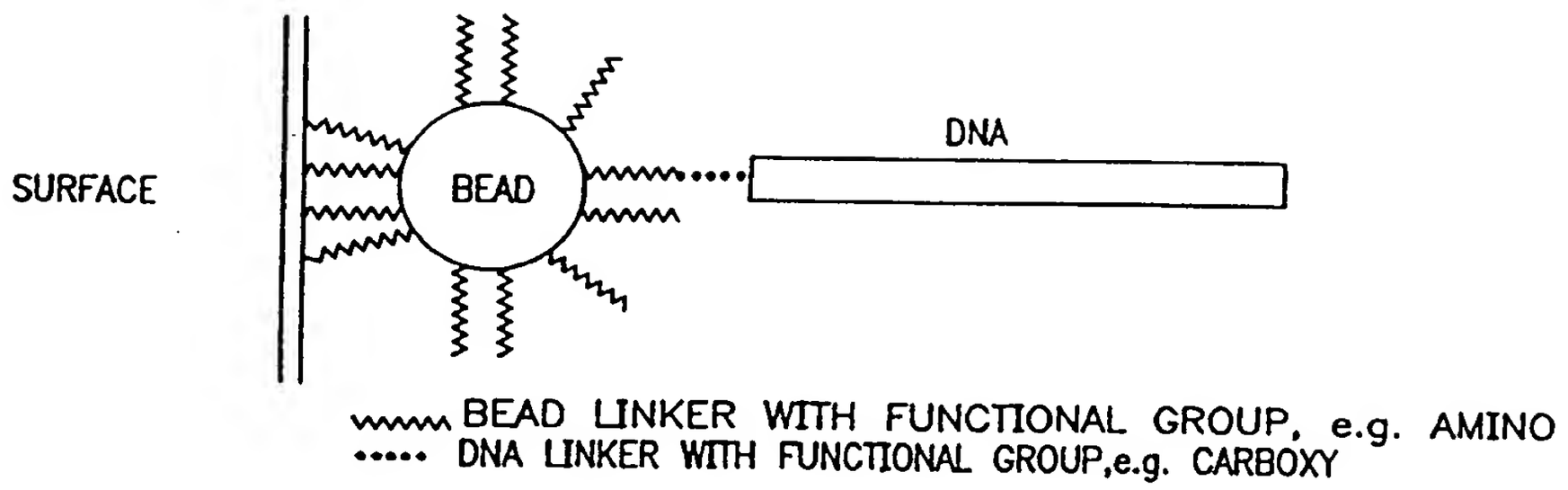

SURFACE
BEAD
DNA
BEAD LINKER WITH FUNCTIONAL GROUP, e.g. AMINO
DNA LINKER WITH FUNCTIONAL GROUP,e.g. CARBOXY

FIG. 1

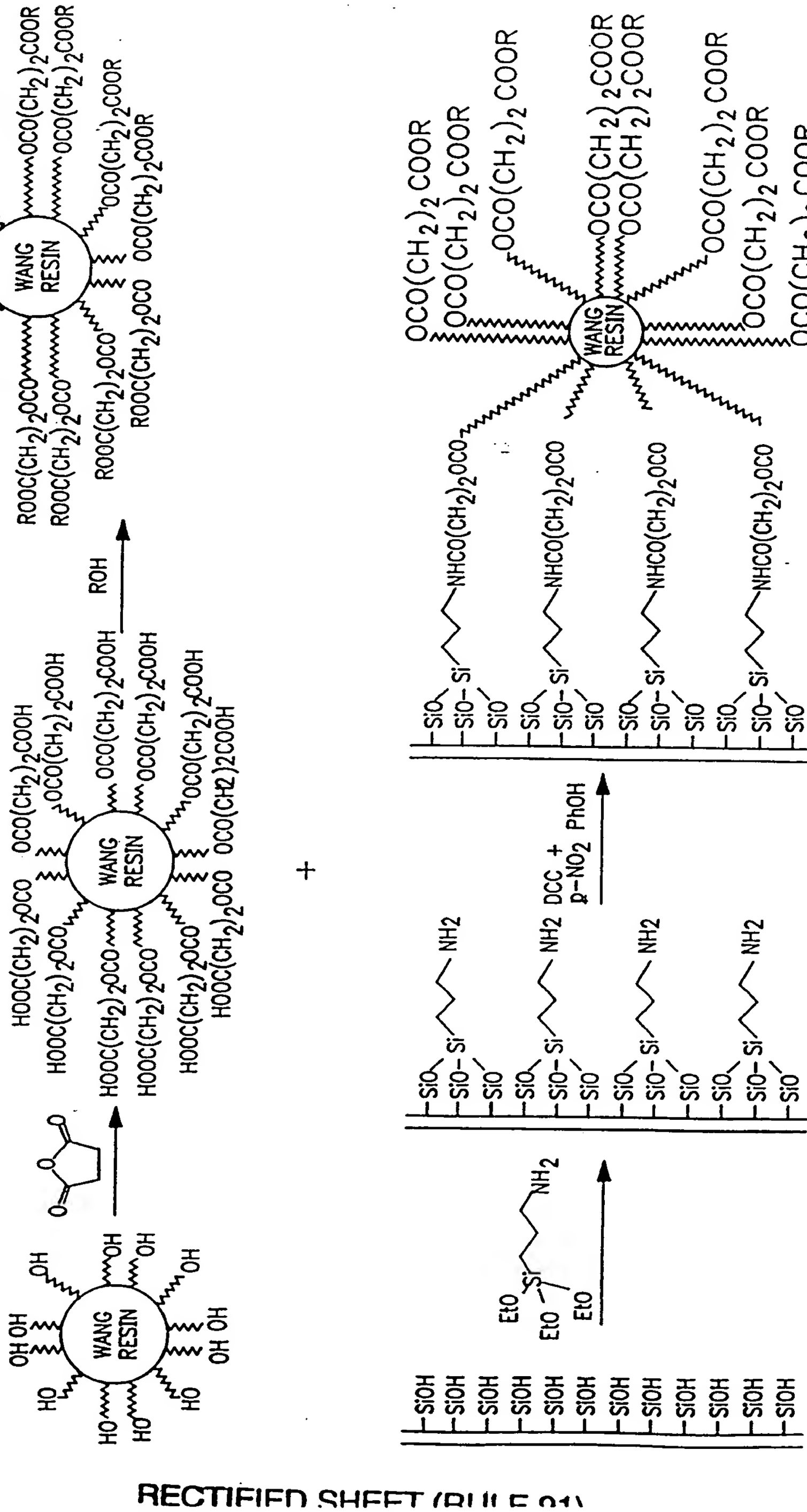
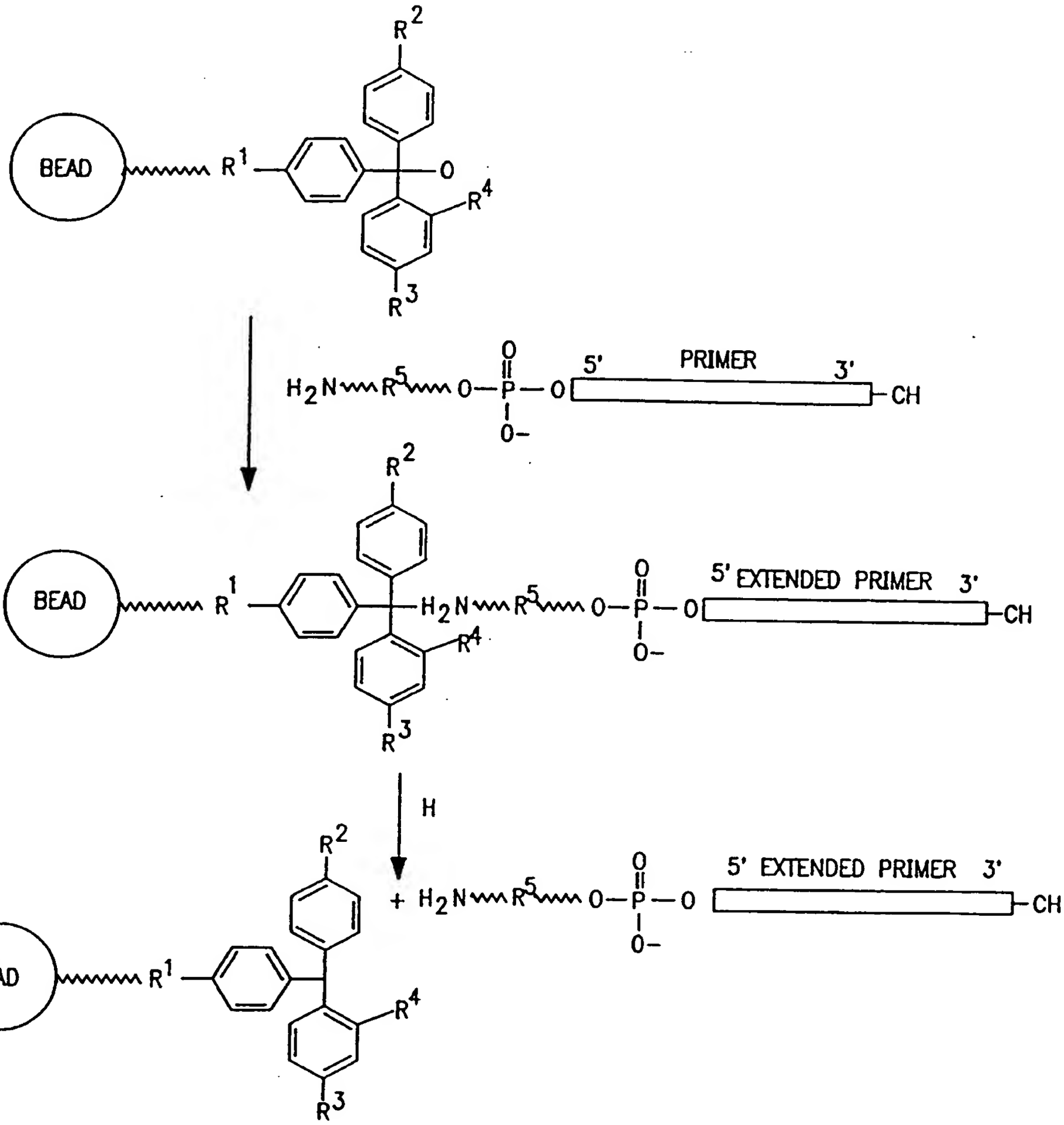

WANG RESIN
ROH
DCC + p-NO2 PhOH
EtO Si EtO EtO
NH2
SiOH
SiO
NHCO(CH2)2OCO
OCO(CH2)2COOR
OCO(CH2)2COOH
ROOC(CH2)2OCO
HOOC(CH2)2OCO
OH
HO

FIG. 2



R^1=COO;(CH_2) ; para or meta)
R^2=MeO;H
R^3=MeO;H
R^4=Cl;N^H
R^5=$(CH_2)_n$; $(CH_2)_nCONH(CH_2)_n$

FIG. 3